Bioserogroups of Campylobacter species isolated from sheep in Kaduna State, Nigeria

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Abstract

Sheep Campylobacter isolates from Kaduna State were characterized into their species and bioserogrouped. A total of 1100 samples were collected from Kaduna abattoir and National Animal Production Research Institute (NAPRI), Shika. The samples were from 250 gallbladder, 250 intestinal contents, 100 fetal stomach contents all from Kaduna abattoir while 250 rectal swabs and 250 vaginal swabs were from the NAPRI Small Ruminant Programme. Of a total of 1100 samples, 39 (3.54%) yielded Campylobacter organisms. The highest isolation rate (6.8%) was from samples of intestinal contents followed by those from gall bladders (4.0%). Samples from the vaginal and fetuses had the lowest isolation rates (2.80%) and (0%), respectively. Of the 39 Campylobacter isolates from all the sources, (79%) were characterized as C. fetus subsp jejuni, C. coli (13%) and C. laridis (8.0%), while C. coli and C. laridis were isolated from gall bladder and intestinal contents only. Campylobacter fetus subsp jejuni biotype I accounted for 40.3% of the total isolates. C. laridis biotypes I and II were also isolated and accounted for 5% and 3% of the isolates, respectively. 5% of the isolates were not typeable. The serogroups 4 (13%), 36 (10%), 9 (10%), 84 (8%), 29 (5%) and 20 (8%) were the commonest serogroups identified in sheep at two locations surveyed. The isolation of Campylobacter organisms from rectum, vagina, gallbladder, and intestinal contents is a clear indication that sheep serves as a reservoir of this organisms in Nigeria. Similarities between documented human Campylobacter isolates in Nigeria and those in the present study raised the possibility of cross-transmission between sheep and man. It is concluded that biotyping and serotyping can be used for epidemiological study of campylobacteriosis due to Campylobacter jejuni in sheep in Kaduna State of Nigeria. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Campylobacter organisms are a leading cause of gastroenteritis in man and animals throughout the world (Lior, 1994). In sheep, campylobacteriosis is characterized by abortion, still births, and birth of weak lambs during late pregnancy (Kimberling, 1988). Campylobacter jejuni and Campylobacter fetus are the causative agents of this disease. The infection is highly contagious and may cause up to 70% of ewes to abort when the organisms are newly introduced into
the flock (Dennis, 1990). Susceptible ewes may acquire infection through ingestion of contaminated Campylobacter organisms with fetal material or uterine discharge. Other sources of infection may include feces of carrier sheep and other mammals (Smibert, 1969).

Several epidemiological studies in developed countries have identified sources of Campylobacter enteritis in man to include animals, food, water, and milk products (Khan, 1982; Skirrow, 1982). Although there are sporadic reports of Campylobacter enteritis in developing countries particularly Nigeria (Olusanyo et al., 1983; Alabi et al., 1986; Ani et al., 1988), very little is known concerning its mode of spread.

A proper understanding of the epidemiology of Campylobacter infections is necessary for the planning of effective prevention and control measures (Adegbola et al., 1990; Raji et al., 1997). Moreover, previous observations of the prevalent biotypes and serogroups of Campylobacter isolates from humans (Alabi et al., 1986), and animal sources, especially poultry and pigs (Olubunmi and Adeniran, 1986; Adegbola et al., 1990), have encouraged us to examine the role of sheep in human Campylobacter enteritis in Nigeria. This paper, therefore, document the isolation, biotyping and serotyping of Campylobacter species isolated from sheep in Nigeria.

2. Materials and methods

A total of 1100 samples were examined from periods of September 1997 to December 1998. These samples were derived from live sheep originating from the National Animal Production Research Institute (NAPRI) Sika-Zaria, and slaughtered sheep from the Kaduna abattoir. The samples comprised of 250 gall bladder contents, 250 intestinal contents, and 100 fetal stomach contents swabs from Kaduna abattoir as well as 250 each of rectal and vaginal swabs from 250 healthy sheep at NAPRI Sika-Zaria.

The samples obtained were inoculated into sterile ferrous bisulphate pyruvate (FBP) broth containing GIBCO nutrient broth which served as transport enrichment medium (TEM). The TEM was brought to the laboratory within 4 h of collection. Butzler’s medium (Oxoid SR 85) supplemented with 7% sheep blood was used as primary isolation medium. After inoculation the plates were incubated using the candle jar extinction method at 37°C for 48–72 h. The suspected colonies were identified by Gram’s stained and biochemical methods as described earlier by Morris et al. (1985) and Billingham (1981).

The colonies were Gram stained and showed small spiral gram-negative bacteria. Organisms were considered to be Campylobacter species if they were motile, catalase positive, oxidase positive, reduced nitrate to nitrite, grew at 37, 42°C, but not at 25°C (Coker and Adefoso, 1994).

Biotyping of Campylobacter isolates was done using the new extended scheme described by Lior (1984). The scheme employs three chemical reactions: rapid hydrogen sulphide production; hippurate hydrolysis; and detection of DNA hydrolysis to differentiate C. jejuni into four biotypes (I, II, III, IV), C. coli into biotypes I and II and C. laridis into biotypes I and II.

Similarly, serotyping of the isolates was done using the scheme of Lior et al. (1982), based on a rapid slide agglutination technique with live bacteria and absorbed antisera for detection of heat labile antigenic factor. The 21 antisera used for this study were supplied by National Reference Service for Campylobacter, Ontario, Ottawa, Canada. The antisera for the C. laridis were not included in the pack.

3. Results

The isolates that were motile gram-negative curved to spiral rods suspected to be Campylobacter were catalase positive, oxidase positive, reduced nitrate to nitrite, did not produce acid or H2S in Triple sugar irons. They grew well at 37 and 42°C but not at 25°C, and also grew in 1% glycine, but not in 3.5% sodium chloride. Those isolates that were hippurate positive but H2S and deoxyribonuclease (DNase) negative were characterized as C. jejuni, while isolates identified as C. coli were hippurate, H2S, and DNase negative. C. laridis isolates were H2S positive, but hippurate and DNase negative.

Table 1 shows the species and biotypes of Campylobacter isolates obtained from different anatomical sites in sheep in Kaduna State.

Of a total of 1100 samples, 39 (3.54%) yielded Campylobacter organisms. The highest isolation rate (6.8%) was from samples of intestinal contents.
followed by those from gall bladders (4.0%). Samples from the vagina and fetuses had the lowest isolation rates (2.80%) and (0%), respectively.

Of the 39 *Campylobacter* isolates from all the sources, (79%) were characterized as *C. fetus subsp. jejuni*, *C. coli* (13%) and *C. laridis* (8.0%). Isolates of *C. coli* and *C. laridis* were obtained from gall bladder and intestinal contents only.

*C. jejuni* isolates from the vagina, gall bladder and intestinal contents were more of biotype I. Whereas, those of rectal swabs were mostly of biotype II. There was no *C. jejuni* biotype IV in the gall bladder, rectal swabs and intestinal contents.

Table 2 shows distribution of different biotypes of *Campylobacter* spp isolated from sheep in Kaduna state. *C. jejuni* biotype I was the most prevalent biotype accounting for (40.3%) of the total isolates while biotype II represented 20.5%. Biotype III 10.2% and biotype IV the least at 8.0%. *C. coli* isolates were of biotypes I and II almost of equal numbers (8%) and (5%), respectively. *C. laridis* were also typed into biotype I and II representing (5%) and (3%),
respectively, from gallbladder and intestinal content only.

Table 3 shows the distribution of different serogroups of Campylobacter species isolated from various anatomical sites of sheep in Kaduna State. For C. jejuni serogroups 36 and 4 were the commonest serogroups representing (10.3%) and (7.7%), respectively. Other serogroups were 29 (5.1%) and 55 (2.6%). C. coli isolates were not serotyped. There was no significant difference in the different anatomical sites for the different serogroups, although serogroup 36 occurred almost in all anatomical sites except in the gall bladder. The serogroups 4 and 9 were the commonest serogroups found in the gall bladder representing 20% each. Other serogroups 53, 20 and 24 occurred in equal proportional 10% each in the gall bladder. Serogroups from the intestinal contents were 20, 84, 9, 36, 4 and 6 representing 11.77% each. The prevalent serogroups from rectal swabs were 18, 36, 22, 45 and 5 representing 14.29% each. Vaginal serogroups were 55, 36 and 29 which occurred in equal proportions of 10% each.

4. Discussion

The result of this study indicate the overall isolation rate of ovine Campylobacter isolates to be 3.54% from all the samples collected. The isolation rate of 2.8% from the rectal swabs of healthy sheep in Zaria were similar to those of Turkson et al. (1988), with the prevalence rate of 2.0% from rectal swabs of healthy sheep in Kenya. Olubunmi and Adeniran (1986) reported a prevalence rate of 6.25% from western Nigeria, and 6.9% prevalence rate reported by Abraham et al. (1990) in healthy Ghanaian sheep.

The results were different from those of Adegbola et al. (1991) and Adetosoye and Adeniran (1987) who
failed to isolate any Campylobacter organism from healthy sheep and goats in their studies in Ile-Ife, Nigeria.

Similarly, all the rectal Campylobacter isolates in this study were C. jejuni with biotype II the commonest representing 57%. This disagreed with the finding of Olubunmi and Adeniran (1986), in which Campylobacter biotype I accounted for 58% of their isolates. The present study agree with Abraham et al. (1990) in which biotype II predominated in their studies.

The isolation of Campylobacter from intestinal contents of sheep at Kaduna abattoir indicated that Campylobacter colonize intestinal mucosa of healthy animals as earlier reported by Butzler and Skirrow (1979). This may serve as a source of infection to other animals in the environment.

The isolation rate of 6.8% is close to the 4.9% reported by Koides (1991) for healthy sheep in Turkey. The two results however differed in the percentages of C. jejuni isolates. In this study, the overall percentage isolation of C. jejuni 64% was much lower than in Koides (1991) work which was 93%. In both findings, other Campylobacter such as C. coli was also isolated from this samples.

The isolation rate of Campylobacter from the vaginal swabs of sheep sampled at the NAPRI farm in Shika Zaria was 2.0%. Although, abortion cases were not reported, the birth of weak lambs was reported by the herdsman. Presence of infection in the vaginal swabs of healthy sheep after parturition was earlier reported by Montagna et al. (1988). In their study just like the present finding, C. jejuni was the major Campylobacter species identified in the vagina of ewes.

Gall bladder regarded as a choice for the isolation of Campylobacter organisms (Firehammer et al., 1962), had a low 4.0% isolation rate in this work. A similar (3.3%) rate was found in the work by Marsh and Firehammer, 1953. This tended to show that most gall bladders of sheep from different geographical locations may have low isolation rate of Campylobacter organisms. In sheep slaughtered at Kaduna abattoir, C. jejuni biotype 1 accounted for 50% of the isolates from the gall bladder.

Most Campylobacter isolates from gall bladder, (67%) in Marsh and Firehammer’s work, 1953 classified as C. fetus subsp jejuni whereas the remaining 33% were C. fetus subsp fetus. In the present study, most gall bladder isolates (80%) were C. jejuni. The others were either C. coli or C. laridis. The differences are not easy to account for but the type of Campylobacter in the gall bladder may be a reflection of the type most commonly isolated in other anatomical parts of the animal. In this study the most common Campylobacter species was C. jejuni.

Failure to isolate Campylobacter from the fetuses examined in this study may be attributed to the fact that the fetuses were not obtained from clinical cases of epizootic abortion outbreaks. The most common biotype of C. jejuni found in the present study was biotype I accounting for 40.3% isolates. This is in agreement with the finding of Adegbola et al. (1990) in Ile-Ife, Nigeria, and Varga et al. (1990) in Hungary. It is different from what Adesiyun et al. (1992) reported. They found C. coli biotype I as the major biotype from the healthy sheep and other farm animals in Trinidad and Tobago. The differences might be due to a number of factors. Part of this may be related to ecologic differences in the areas where the studies was conducted. Similarly, the laboratories methods used in different studies could have influenced results obtained as shown by the works of Adetosoye and Adeniran (1987), and Adegbola et al. (1991) as compared with that of Olubunmi and Adeniran (1986), in the same locality but in different laboratories.

Campylobacter fetus known to colonize the intestinal tract of domestic animals (Doyle, 1981; Manser and Dalziel, 1985), were not isolated in the present study. This may be due to hot climatic conditions in this part of the country, since Campylobacter fetus survive well in areas with lower temperature, especially at 25–37°C.

The isolation of Campylobacter organisms from rectal, vaginal, intestinal content, gall bladder swabs in this study is a clear indication of the presence of Campylobacter in sheep in Zaria and Kaduna. Campylobacter organisms must be considered as potential agents of Ovine enteritis and abortion in Nigeria.

The most common serogroups of Campylobacter sp. isolated from sheep in this present study were 4, 36, 20, 84, 9, 29, 6, and 8 accounting for 64% of the total isolates. Adegbola et al. (1991) reported serogroups 2, 4, 29 and 36 which accounted for 61.7% of all the serogroups as the major ones found in animals in Ile-Ife. In another study, Adegbola et al. (1990) reported serogroups 2, 4, 20, 29, 36 and 45 to be
predominant among animals in Ile-Ife. In that study they also found serogroups 2, 4, 29, 36, 45 and 53 to be the most common in humans. Recently, Coker and Adefoso (1994) reported that during a 10 years study, serogroups 29 and 36 were the commonest serogroups in human in Lagos.

The serogroups found in sheep in this study were similar to those reported in domestic animals previously in Nigeria (Adegbola et al., 1990, 1991) and in humans (Alabi et al., 1986; Coker and Adefoso, 1994).

During slaughter of sheep, goats and cattle, intestinal feces often contaminate meat. Meat and meat products should therefore, be properly cooked to destroy the organism so as to prevent possible transmission to man.

It is well established that sheep are natural reservoir of Campylobacter organisms (Firehammer et al., 1962). The serotypes identified in sheep in this study are similar to those reported previously in humans in Nigeria, which suggests the possibility of cross-transmission of Campylobacter from sheep to man. To our knowledge this may be the first time common serotypes are found in the northern part of Nigeria from sheep.

5. Conclusion

Results from this study indicate that C. jejuni, C. coli and C. laridis are the most common species of Campylobacter organisms isolated from healthy sheep in Zaria and Kaduna. It is concluded that biotyping and serotyping can be used for epidemiological study of Campylobacteriosis due to Campylobacter species in sheep in Kaduna State, Nigeria.

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References


